hindrance can be overcome by combining, as a spacer, nucleotides with no steric hindrance such as deoxyadenylic acid (dAMP), deoxycytidylic acid (dCMP), deoxyguanylic acid (dGMP), thymidylic acid (dTMP), or deoxyuridylic acid (dUMP). However, the DNA resulting from the reaction could have a different sequence from that of the original one because of the introduced nucleotide compounds and/or the spacer nucleotides. The sequence derived from the nucleotide compounds added can cause additional hybridization, which can be a problem in some cases. An example of nucleotide sequences causing such hybridization is a sequence where a single type of nucleotide is consecutively repeated, which is represented by a poly(A) tail of cDNA. If the signal/noise ratio (hereafter, referred to as S/N ratio) is decreased because of hybridization due to the sequences of nucleotides newly added for labeling, then the sensitivity is not increased even when the specific activity of the probe is higher.

In order to inhibit hybridization due to the sequence of nucleotides newly added for labeling but not derived from the DNA to be labeled, it is necessary to use, as a carrier in the hybridization, homopolymer or random copolymer of deoxyadenylic acid (dAMP), deoxycytidylic acid (dCMP), deoxyguanylic acid (dGMP), thymidylic acid (dTMP), and/or deoxyuridylic acid (dUMP) and to mask the nucleotide sequence responsible for the high levels of background around the signal. Nonetheless, even by this method, it is hard to completely inhibit such hybridization due to the sequences of nucleotides newly added for labeling.

In the procedure for gene expression profiling using current array technologies, cDNA corresponding to an RNA to be tested is labeled and then hybridized to immobilized cDNA or oligo DNA (reverse Northern blotting; Science (1999), 283, 83-87; Nat Biotechnol (1996), 14, 1675-80). Unlike ordinary Northern blotting, this method requires not a labeled probe but a labeled target nucleotide sequence, because it is difficult to achieve sufficient sensitivity in this type of analytical method based on current labeling techniques. This method is excellent to analyze a large number of genes at once. However, when the alteration in expression level is to be determined based on the variation of signal intensity between different arrays,

such arrays to be compared must be identical. Actually, it is difficult to secure such uniformity among multiple arrays. Therefore, it is necessary to normalize the difference between arrays, if two or more types of RNAs are to be compared with each other with different arrays. Thus, when there are two types of RNAs to be compared, they are typically labeled with different fluorescent compounds and competitively hybridized to a single array to observe the alterations in expression level. Probes achieving sufficient sensitivity makes it possible to employ the same principle as used for the usual Northern hybridization, namely a method in which a labeled probe can be allowed to react to immobilized target nucleotide sequences. If such a method is established, the expression levels of multiple genes can be analyzed with a single array, which is expected to potentate the applicability of array technology increasingly.

It is known that inosinic acid can be used as a substrate in the nucleotide amplification reaction to prevent secondary structure formation of amplification products. Formation of the secondary structure is considered to result in the reduction of resolution, e.g., in gel electrophoresis. Thus, it can be expected that the application of inosinic acid improves the sensitivity. This principle has been applied to PCR for DNA synthesis (Proc. Natl. Acad. Sci. USA., 76, 2232-2235) and a system of RNA synthesis (Unexamined Published Japanese Patent Application (JP-A) No. Hei 6-165699. It has also been reported that the application of 7-deaza-2'-deoxyguanosine-5'-triphosphate (c7dGTP) prevents the formation of complicated secondary structure in PCR (WO90/03443). However, the applications shown in these reports are achieved merely for the purpose of inhibiting secondary structure formation in nucleotide amplification products, but are not construed as suggesting the prevention of nonspecific base pairing in hybridization assays. In the structural aspect, these methods are different from the method of the present invention in that some portions were converted to inosine in the nucleotide sequence of target nucleic acids in the previous methods.

Disclosure of the Invention

An objective of the present invention is to provide a labeled DNA that meets the two criteria of both high specific activity and high specificity.

The present inventors have noticed that one of causes for the reduction of hybridization specificity of a DNA labeled by 3'-tailing is originating from sequences of nucleotides or nucleotide compounds added for labeling. As long as the nucleotide sequence consists of a, c, t, g, and u, the nucleotide sequence added for labeling can result in occurrence of hybridization with a nucleotide sequence complementary to the nucleotide sequence. Then, the inventors considered the possibility that the specificity is improved by using nucleotides and nucleotide compounds exhibiting only weak affinity in base pairing as nucleotides and nucleotide compounds to be added for labeling. Further, the present inventors selected nucleotides and nucleotide compounds as substrates for terminal transferase to establish a 3'-tailing label method capable of providing probes having high specific activity, and thereby completed the present invention. Specifically, the present invention relates to the labeled DNA, the method for providing the labeled DNA, and the use thereof as follows:

(1) a hybridization probe in which a nucleotide sequence comprising labeled nucleotides or nucleotide derivatives is added to a DNA to be labeled, the added nucleotide sequence

a) comprising nucleotides and/or nucleotide derivatives having weaker affinity of hydrogen bonding in base pairing with bases of the target nucleotide sequence when compared with those of hydrogen bonding in an a/t pair, in an a/u pair, and in a g/c pair; and

b) being introduced into the DNA to be labeled through nucleotide-adding reaction with terminal transferase;

(2) the hybridization probe of (1), wherein the nucleotides of a) are inosinic acids;

(3) the hybridization probe of (2), wherein the added nucleotide sequence comprises labeled nucleotides or nucleotide derivatives and unlabeled inosinic acids or derivatives thereof;

(4) the hybridization probe of (3), wherein the labeled nucleotides or nucleotide derivatives are labeled inosinic acids or